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Phylogenetics of *Ruscaceae* sensu lato based on plastid *rbcL* and *trnL-F* DNA sequences

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Abstract: JANG C.G. & M. PFOSSER (2002): Phylogenetics of *Ruscaceae* sensu lato based on plastid *rbcL* and *trnL-F* DNA sequences. — *Stapfia* 80: 333-348.

We studied the phylogeny and relationships among several families within Asparagales by analysis of *trnL-F* and *rbcL* sequences. As judged from *rbcL*, *trnL-F* or combined data, the order Asparagales is monophyletic with high bootstrap support. The classification into higher and lower asparagoids, which are characterized by successive microsporogenesis and simultaneous microsporogenesis, respectively, is only weakly supported by the *trnL-F* and combined data. The lower asparagoids with simultaneous microsporogenesis are clearly not monophyletic. Within Asparagales, the families *Convallariaceae*, *Ruscaceae*, *Dracaenaceae* and *Nolinaceae* are not monophyletic. This is well supported by *trnL-F* data and the combined analysis and moderately supported by *rbcL* sequences. Especially *Convallariaceae* are highly polyphyletic. However, the four families formed a clearly defined clade in our analysis and thus, the concept of lumping the four families into a large family *Ruscaceae* s. lat. received further support. Within *Ruscaceae* s. lat., the genus *Peliosanthes* does not have a fixed position. This genus occupies a position basal to the remaining taxa both in the *rbcL* and the combined trees, but falls into a clade together with *Liriope* and *Ophiopogon* like in traditional taxonomy in the *trnL-F* analysis. Both in the *trnL-F* and the combined trees the families *Asparagaceae* and *Eriospermaceae* are located next to *Ruscaceae* s. lat., but no clear sister group relationship is obvious from our data. Lumping of *Eriospermaceae* with *Ruscaceae* s. lat. is not supported by any of the molecular data sets.

Zusammenfassung: JANG C.G. & M. PFOSSER (2002): Die Phylogenie der *Ruscaceae* sensu lato - analysiert mit Hilfe von *rbcL* und *trnL-F* DNA-Sequenzen. — *Stapfia* 80: 333-348.

Wir untersuchten in dieser Arbeit die phylogenetischen Verwandtschaftsbeziehungen innerhalb der Ordnung Asparagales. Aufgrund von *rbcL* und *trnL-F*-Daten ist die Ordnung Asparagales monophyletisch. Die Unterscheidung zwischen "higher asparagoids" (sukzessive Mikrosporogenese) und "lower asparagoids" (simultane Mikrosporogenese) wird nur schwach durch die molekularen Daten unterstützt. Die "lower asparagoids" stellen auf alle Fälle keine monophyletische Gruppe dar. Innerhalb der Asparagales sind die Familien *Convallariaceae*, *Ruscaceae*, *Dracaenaceae* und *Nolinaceae* nicht monophyletisch. Vor allem sind die *Convallariaceae* deutlich polyphyletisch. Allerdings bilden diese vier Familien zusammen eine gut abgesicherte monophyletische Gruppe, wodurch das Konzept, diese Familien zu einer großen Familie *Ruscaceae* s. lat. zusammenzuziehen, weiter unterstützt wird. Innerhalb der Familie *Ruscaceae* s. lat. nimmt die Gattung *Peliosanthes* eine labile Position ein. Diese Gattung steht in der *rbcL* und der kombinierten Analyse basal zu den übrigen Gattungen, im *trnL-F* Baum bildet sie ähnlich wie in der traditionellen Klassifikation mit *Liriope* und *Ophiopogon* einen Clade. Sowohl in der *trnL-F* als auch in der kombinierten Analyse sind die Familien *Asparagaceae* und *Eriospermaceae* die nächst verwandten Familien zu *Ruscaceae* s. lat. Eine Eingliederung der *Eriospermaceae* in *Ruscaceae* s. lat. wird durch die molekularen Daten nicht unterstützt.

Key words: Asparagales, *Ruscaceae* s. l., *Peliosanthes*, *trnL-F*, *rbcL*.

Introduction

Based on the important synapomorphy of their phytomelanin-containing seed coat, HUBER (1969) was the first who identified the order Asparagales as asparagoid Liliiflorae (=Lilianeae). DAHLGREN & RASMUSSEN (1983) and DAHLGREN et al. (1985) regarded the order Asparagales as a monophyletic group by HUBER's and several other characters. The Asparagales consist of a paraphyletic "lower" asparagoid assemblage consisting of the phylogenetically early branching lines, such as the *Orchidaceae* and the related families *Asteliaceae*, *Blandfordiaceae*, *Boryaceae*, *Hypoxidaceae* and *Lanariaceae*; the *Iridaceae* with the related families *Doryanthaceae* and *Ixioliriaceae*; the *Asphodelaceae* with the related families *Hemerocallidaceae* and *Xanthorrhoeaceae*; and the *Tecophilaeaceae*. A more or less monophyletic "higher" asparagoid clade includes the *Agavaceae*, *Alliaceae*, *Amaryllidaceae*, *Behniaceae*, *Convallariaceae*, *Hyacinthaceae* and *Laxmanniaceae* (*Lomandraceae*). This phylogenetic grouping was supported by both molecular and morphological evidence (CHASE et al. 1995; RUDALL & CUTLER, 1995; RUDALL et al. 1997; CONRAN & TAMURA, 1998; FAY et al. 2000; CHASE et al. 2000a). Although most monocots usually have successive microsporogenesis, simultaneous microsporogenesis with inferior ovaries are characteristic of 'lower' asparagoids with a few exceptions. All 'higher' asparagoids have successive microsporogenesis and mostly have superior ovaries (RUDALL et al. 1997).

Although DAHLGREN et al. (1985) and TAKHTAJAN (1997) have considered the *Ruscaceae* s. str. most closely related to *Asparagaceae*, the relationship between *Ruscaceae* and *Asparagaceae* is still doubtful. The two families show several similarities, e. g. in sharing phylloclades and having baccate fruits and similar karyotypes (SATO, 1942; SEN, 1978; TAMURA, 1995). However, differences between *Ruscaceae* and *Asparagaceae* are found in the phylloclades of *Ruscaceae* being leaf-like and those of *Asparagaceae* being stem-like, in shoot architecture, seed coat anatomy, and in the position of inflorescences (CONRAN & TAMURA, 1998). The homology of the phylloclades between *Ruscaceae* and *Asparagaceae* has been questioned by several studies like those of ARBER (1924), SCHLITTLER (1953) and COONEY-SOVETTS & SATTLER (1986). COONEY-SOVETTS & SATTLER (1986) regarded the phylloclades as intermediate structure between shoot and leaves. RUDALL et al. (1998) regarded the karyotype of *Ruscaceae* ($x = 20$) more similar to *Convallariaceae* s. lat. (mostly $x = 18, 19$) than *Asparagus* ($x = 10$). The lack of phytomelanin in the seed coat of *Ruscaceae* is shared with *Convallariaceae* s. str., but not with *Asparagaceae*. Serologically, the *Ruscaceae* are closer to *Convallariaceae* than to *Asparagus* (CHUPOV & CUTJAVINA, 1980). On the other hand, CHASE et al. (1995) suggested a close relationship between *Ruscaceae*, *Convallariaceae*, *Nolinaceae*, *Dracaenaceae* and *Comospermum*. In all cases known so far, molecular data place *Ruscaceae* at some distance from *Asparagaceae*.

The *Nolinaceae* are usually positioned near *Dracaena* in a broadly defined family *Liliaceae*. They have been placed in the tribe *Dracaeneae* (BENTHAM & HOOKER, 1883), or *Nolineae* (KRAUSE, 1930). Later, *Nolinaceae* were included in *Agavaceae* by HUTCHINSON (1959), but this placement is neither supported by karyological data, nor by flower, fruit or seed morphology. The members of *Nolinaceae*, such as *Dasyllirion*, *Nolina*, *Beaucarnea* and *Calibanus*, have a basal chromosome number of $n = 19$, similar in size and number to the karyotypes of *Dracaena*, *Sansevieria* and

Liriope (Convallariaceae) (SHARMA & CHAUDHURI, 1964). Consequently, in DAHLGREN et al.'s system (1985) the *Nolinaceae* were excluded from *Agavaceae* and positioned next to *Dracaenaceae*. These two families are placed near *Convallariaceae* and *Asparagaceae*. A close relationship between *Ruscaceae*, *Dracaenaceae* and *Nolinaceae* was suggested by *rbcL* sequence data (CHASE et al. 1993; DUVAL et al. 1993) and was also supported by cytological data (TAMURA, 1995). CONRAN (1989) placed *Ophiopogon* close to the clade of *Ruscaceae/Asparagaceae*, which was partially supported by the molecular study of CHASE et al. (1995). *Liriope* and *Peliosanthes* were placed at some distance to each other, with *Liriope* being closer to *Nolina* and *Calibanus* (*Nolinaceae*), *Aspidistra* (*Convallariaceae*), *Danae* (*Ruscaceae*) and *Comospermum* than to *Peliosanthes* and *Polygonatum* (*Convallariaceae*). In the molecular study of CHASE et al. (1995), the *Convallariaceae* were treated as a paraphyletic assemblage together with *Nolinaceae*, *Ruscaceae*, *Dracaenaceae* and *Comospermum*. The genera formerly included within *Convallariaceae* are embedded within a larger, robust and well supported clade for which generic and familial level relationships were hitherto largely unresolved. In many following studies, the monophyly of *Convallariaceae* s. lat. (in the sense of CHASE et al. (1995)) was strongly supported (RUDALL et al. 1997; APG, 1998; RUDALL et al. 2000; YAMASHITA & TAMURA, 2000).

DAHLGREN et al. (1985) mainly used plesiomorphic characters to discriminate *Convallariaceae* from other members of Asparagalean families such as, baccate fruits, non-phytomelaniferous seed coat. The *Convallariaceae* sensu DAHLGREN et al. (1985) are polyphyletic with *Nolinaceae*, *Ruscaceae*, *Dracaenaceae* and *Comospermum* in the study of RUDALL et al. (1997).

Therefore, a large family *Ruscaceae* s. lat. was proposed by RUDALL et al. (2000) based on a combined molecular-morphological analysis, which includes *Convallariaceae*, *Nolinaceae*, *Ruscaceae*, *Dracaenaceae*, *Comospermum*, and *Eriospermum*. *Ruscaceae* SPRENGEL (1826) has nomenclatural priority to *Convallariaceae* HORANINOW (1834). RUDALL et al. (2000) suggested to change the name *Convallariaceae* s. lat. to *Ruscaceae* s. lat. This clade *Ruscaceae* s. lat. consists of 6 groups: (1) *Eriospermum*, (2) *Comospermum*, (3) nolinoids (former *Nolinaceae*) together with *Ophiopogon* and *Liriope* (*Ophiopogoneae*, excluding *Peliosanthes*), (4) dracaenoids (former *Dracaenaceae*), (5) *Polygonatae* and (6) a group consisting of *Convallariaceae* (including *Aspidistra*) and the ruscoids (*Ruscaceae* s. str.) plus *Peliosanthes*. This grouping is well supported by molecular data (FAY et al. 2000) and further substantiated by the absence of phytochrome in the seed coat.

The *Asparagaceae* were often considered as the sister group of *Ruscaceae* based on several aspects, such as cytological (TAMURA, 1995) and morphological evidence. However, some molecular studies indicated *Eriospermum* and/or *Aphyllanthes* to be possible sister groups to *Ruscaceae* (FAY et al. 2000; YAMASHITA & TAMURA, 2000).

In this study, we used sequence comparisons of *trnL* intron and *trnL-trnF* intergenic spacer sequences (IGS) as well as *rbcL* data of the chloroplast genome to provide an independent assessment of systematic relationships within Asparagales, with emphasis on *Ruscaceae* s. lat.. The *trnL* intron and *trnL-F* IGS regions have been proven to be particularly phylogenetically informative in several other studies (HAM et al. 1994, KIM et al. 1996), *Gentianaceae* (GIELLY & TABERLET, 1996), *Rubiaceae* (ROVA et al. 1997), *Alliaceae* (FAY et al. 1997), *Dipterocarpaceae* (KAJITA et al. 1998),

Monimiaceae (RENNER, 1998), *Hyacinthaceae* (PFOSSER & SPETA, 1999), with sequence divergence rates considerably higher than in *rbcL* (GIELLY & TABERLET, 1994). In plants, the *trnL* intron usually shows sequence conservation in the regions flanking both *trnL* exons (BAKKER et al. 2000), but substitution rates for the *trnL-F* IGS are sometimes higher than those of the *trnL* intron for several plant groups. The plastid *rbcL* gene has a three times slower rate of divergence. However, it was shown that combining *rbcL* and *trnL-F* data can improve the resolution of phylogenetic trees (MEEROW et al. 1999; CHASE et al. 2000b).

This study was undertaken to address six main points: (1) to test the monophyly of *Ruscaceae* s. lat.; (2) to unravel the intrafamilial relationships of *Ruscaceae* s. lat.; (3) to find the possible sister group for *Ruscaceae* s. lat.; (4) to confirm the split of the Asparagales into 'lower' and 'higher' asparagoids; (5) to prove the monophyly of the order Asparagales; (6) to evaluate the impact of *trnL-F* phylogeny for the classification of Asparagales, especially *Ruscaceae* s. lat.

Material and Methods

Taxa Sampled. This study is based on plant material collected in the field or taken from the living plant collection of the Botanical Garden of Vienna University. To complete the sampling, data from the EMBL database have been retrieved for those taxa where living plant material was not available for sequencing. Voucher information for all plant accessions, geographic origin, and EMBL database accession numbers are provided in Appendix 1. Nomenclature and familial treatment follows that of CONRAN & TAMURA (1998), except for taxa which have been recognized both at specific or subspecific levels. In such cases preference was given to the specific treatment.

DNA Extraction. Total genomic DNA was extracted from silica gel-dried and powdered leaf material in 700 µl CTAB buffer (2% CTAB, 100 mM Tris, 1.4 M NaCl, 20 mM EDTA, 0.2% mercaptoethanol, pH 8.0) for 30 minutes at 60°C. 500 µl chloroform/isoamylalcohol (24/1) were added and the extraction mixture was incubated for 15 minutes at 4°C. After centrifugation, the DNA was precipitated with 500 µl isopropanol. The pellet was washed with 70% ethanol and dissolved in 100 µl TE buffer.

DNA Sequencing. Two non-coding regions of the chloroplast genome were sequenced. The *trnL*(UAA) intron and the intergenic spacer (IGS) between the *trnL*(UAA)-3' exon and the *trnF*(GAA) gene were amplified together in a single PCR reaction (PFOSSER & SPETA 1999). Amplified double-stranded DNA fragments were sequenced directly on an ABI377 automated sequencer (Perkin Elmer, UK) following the DYEnamicET cycle sequencing protocol (Amersham Pharmacia, USA). Both strands were sequenced using nested sequencing primers (PFOSSER & SPETA 1999). On average, less than 1% of data matrix cells are scored as missing data. The *rbcL* data were retrieved from the EMBL nucleotide database.

Phylogenetic Analysis. Sequence manipulations were performed on a Digital Alpha 1000A 5/400 server under the operating system Digital Unix V.4.0D. DNA sequences

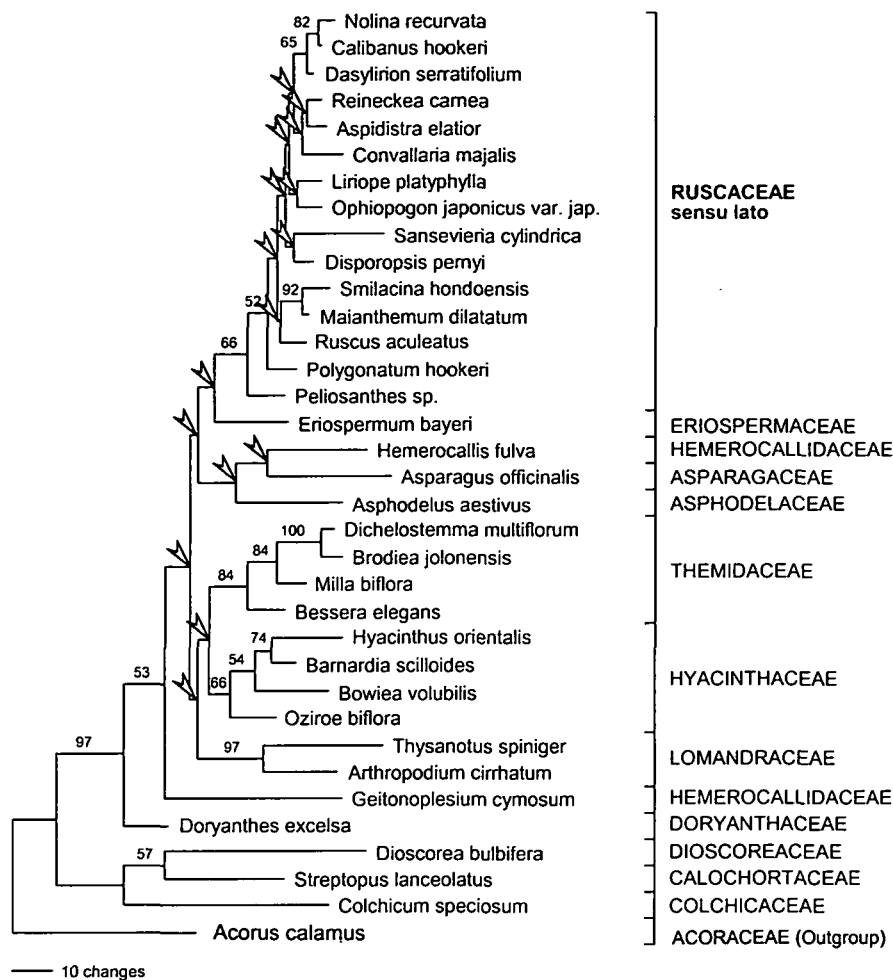


Fig. 1: Maximum parsimony tree based on *rbcL* sequences of representatives from 12 families from the orders Asparagales and Liliales. The tree is rooted with *Acorus calamus*. Bootstrap values 50% are indicated above branches. Nodes not present in the strict consensus tree are marked with arrows. Familial groupings in the sense of APG (1998), CHASE et al. (2000), FAY et al. (2000) are indicated on the right margin.

were pre-aligned using the PileUp program of the GCG software package (Genetics Computer Group 1994) and trimmed on both ends to compensate for different sequence lengths. Final alignment of DNA sequences was done visually. The sequences have been trimmed on both ends to exclude ambiguous positions in close proximity to the sequencing primers. All sequences have been deposited in the EMBL database (for accession numbers refer to Appendix 1). Phylogenetic analysis using the maximum parsimony (MP) method was performed with the computer program PAUP version 4.0b4 (SWOFFORD 2000). Most parsimonious trees were obtained by 1000 replicates of random sequence addition using tree bisection-reconnection (TBR) branch swapping under the Fitch criterion (FITCH 1971). Ten thousand fast bootstrap replicates (FELSENSTEIN 1985) were used to assess confidence limits for the resulting tree topologies. Tree manipulations were performed using MacClade version 3.06 (MADDISON & MADDISON 1992).

Results

DNA sequence variation. The aligned *rbcL* sequences yielded a data matrix of 1440 nucleotides (nt). No insertions/deletions were found resulting in an unambiguous alignment for the *rbcL* sequences. The *trnL-F* (*trnL* intron and *trnL-F* intergenic spacer) sequences in this study varied in length from 842 to 986 nucleotides (nt) resulting in an aligned data matrix of 1451 nt. The length of the combined *rbcL+trnL-F* sequence matrix was 2891 nt. Several insertions/deletions (indels) and length variations of mononucleotide repeats were found in the *trnL-F* region. Since the alignment in these regions was not always unambiguous, such regions have been excluded from the analysis. All sequences have been trimmed on both ends to exclude ambiguities resulting from sequencing artefacts.

Phylogenetic analysis of *rbcL* data: Maximum parsimony analysis under the Fitch criterion of *rbcL* data produced more than 500 equally parsimonious trees with a tree length of 737 steps, a consistency index (CI) of 0.579, a retention index (RI) of 0.553 and a rescaled consistency index (RC) of 0.321. One of the equally most parsimonious trees is shown in Fig. 1. The order Asparagales appeared as a well-supported monophyletic group with a high bootstrap support value (97%). Within Asparagales, no evidence for discrimination into higher and lower asparagoids was found in the molecular data. Based on bootstrap support values of *rbcL* data alone, no direct sister group relationship between *Ruscaceae* s. lat. and any other family within Asparagales was visible. The family *Ruscaceae* s. lat. was monophyletic. However, this clade received only moderate bootstrap support (66%). Within *Ruscaceae* s. lat. the genus *Peliosanthes* occupied the most basal position. Genera, previously included within the families *Ruscaceae* s. str., *Convallariaceae*, *Dracaenaceae* and *Nolinaceae* appeared completely intermixed.

Phylogenetic analysis of *trnL-F* data: Fitch analysis of *trnL-F* data produced 53 trees of 1367 steps with a CI of 0.744, a RI of 0.782 and a RC of 0.581. Compared to the *rbcL* topology, the order Asparagales was monophyletic in the *trnL-F* data but received a lower bootstrap support (74%). Within Asparagales, the higher asparagoids were monophyletic with weak bootstrap support (59%). The lower asparagoids, represented by the families *Doryanthaceae*, *Hemerocallidaceae* and *Asphodelaceae* were paraphyletic in our analysis. The former *Ruscaceae* s. str., *Convallariaceae*, *Dracaenaceae* and *Nolinaceae* formed a monophyletic clade, which received 100% bootstrap support. There was only a weak indication (53%) for a sister group relationship of either *Eriospermaceae* or *Asparagaceae* to *Ruscaceae* s. lat. Within *Ruscaceae* s. lat., the tribe Ophiopogoneae (*Liriope*, *Peliosanthes*, *Ophiopogon*) formed a moderately supported monophyletic group (66%).

Phylogenetic analysis of combined *rbcL* and *trnL-F* data: Maximum parsimony analysis of the combined *rbcL+trnL-F* matrix yielded 82 Fitch trees of 2114 steps with a CI of 0.683, a RI of 0.753 and a RC of 0.514. The topology of the combined tree largely followed that of the *trnL-F* analysis and the two data sets complemented each other well. The order Asparagales appeared as a well-supported monophyletic group with a higher bootstrap support (99%) than in either of the separate analyses (97% and 74% in the *rbcL* and *trnL-F* analysis, respectively). Within Asparagales, the higher asparagoids were monophyletic with weak bootstrap support (63%). The lower asparagoids were paraphyletic like in the *rbcL* analysis. The sister group relation-

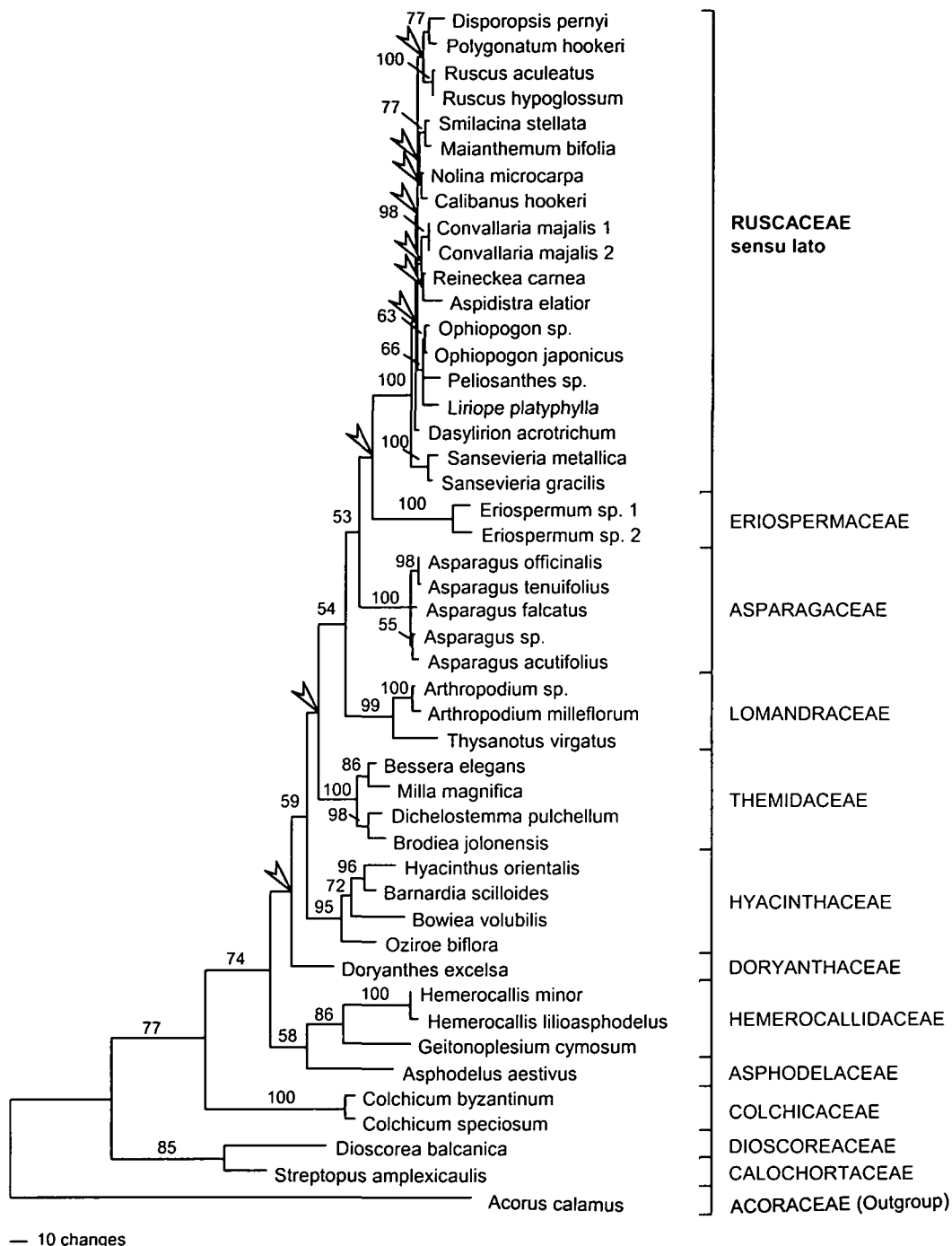


Fig. 2: Maximum parsimony tree based on *trnL-F* sequences of representatives from 12 families from the orders Asparagales and Liliales. The tree is rooted with *Acorus calamus*. Bootstrap values 50% are indicated above branches. Nodes not present in the strict consensus tree are marked with arrows. Familial groupings in the sense of APG (1998), CHASE et al. (2000), FAY et al. (2000) are indicated on the right margin.

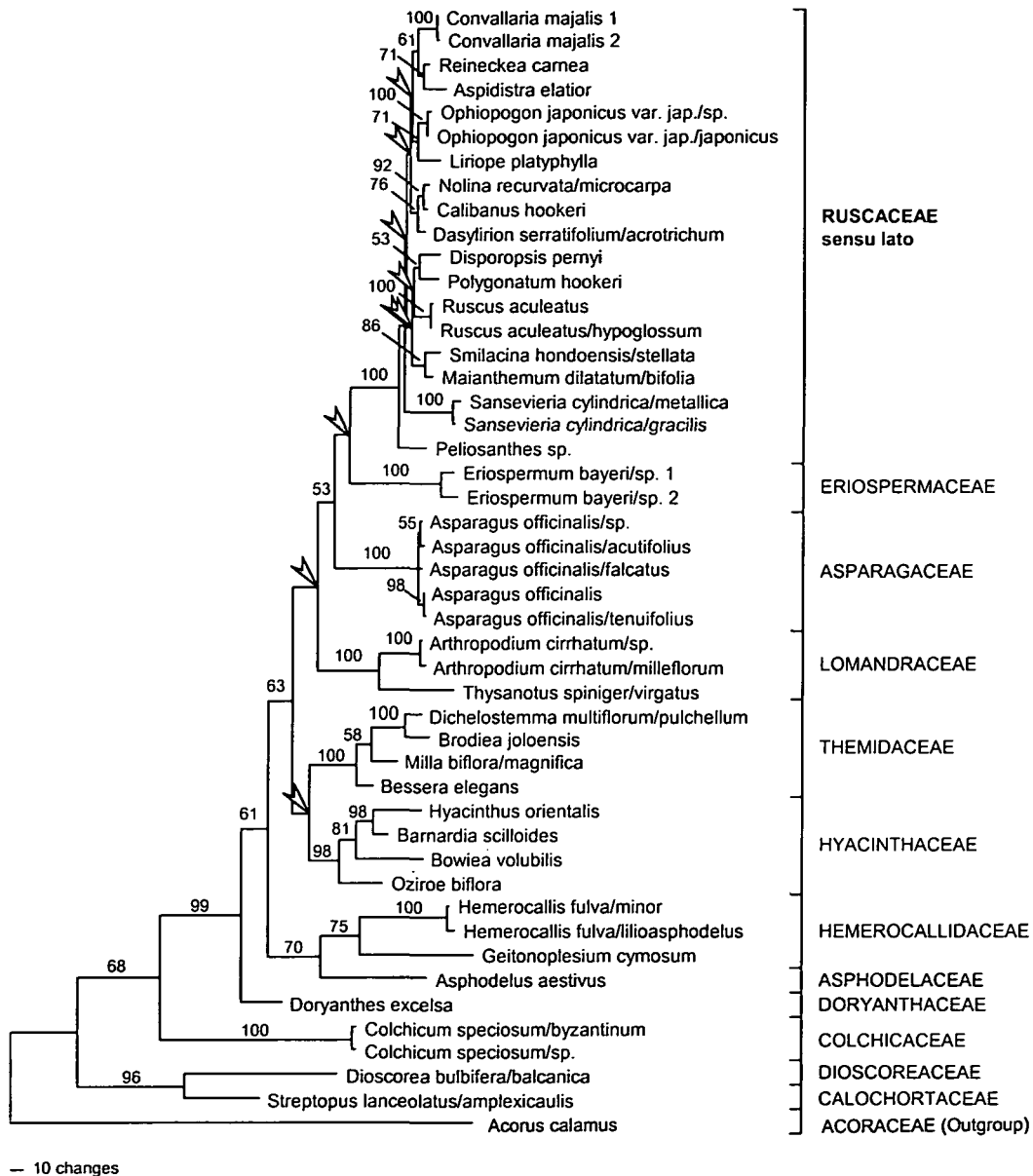


Fig. 3: Maximum parsimony tree based on a combined analysis of *rbcL*+*trnL-F* sequences of representatives from 12 families from the orders Asparagales and Liliales. When sequences of different species have been combined, both names were used to label the terminal nodes of the tree. The tree is rooted with *Acorus calamus*. Bootstrap values 50% are indicated above branches. Nodes not present in the strict consensus tree are marked with arrows. Familial groupings in the sense of APG (1998), CHASE et al. (2000), FAY et al. (2000) are indicated on the right margin.

ship of *Ruscaceae* s. lat. was not resolved. *Asparagaceae* and *Eriospermaceae* were the families most closely related to *Ruscaceae* s. lat. However, this topology was only weakly supported by bootstrap values (53%). Also in the combined analysis, there was strong support for a monophyletic lineage including *Ruscaceae* s. str., *Convallariaceae*, *Dracaenaceae* and *Nolinaceae* (100%). Within *Ruscaceae* s. lat.,

tribe Ophiopogoneae was not monophyletic. The position of the genus *Peliosanthes* was basal like in the *rbcL* analysis.

Discussion

Our data further support the monophyly of the order Asparagales sensu DAHLGREN et al. (1985). In our study, this clade received high bootstrap support in all trees. The order Asparagales consists of a monophyletic higher asparagoid clade which is characterized by successive microsporogenesis and generally superior ovaries and a paraphyletic lower asparagoid clade with simultaneous microsporogenesis and inferior ovaries (CHASE et al. 1995; RUDALL et al. 1997; CONRAN & TAMURA, 1998). Successive microsporogenesis is common in monocotyledons with some exceptions and only the lower asparagoids can be characterized as a group by simultaneous microsporogenesis (RUDALL, 1997). However, based on molecular data, splitting of the Asparagales into higher and lower asparagoids is questionable since monophyly of either of the two groups is missing or supported by low bootstrap values only.

The *rbcL* tree alone did not identify a sister group for *Ruscaceae* s. lat., but the *trnL-F* and the combined analysis were more or less consistent with some previously published analyses (RUDALL et al. 1998, Fay et al. 2000). Such molecular studies usually placed the family *Asparagaceae* as the sister of *Ruscaceae* s. lat. The genus *Eriospermum* was also found in other studies to be closely related to *Ruscaceae* s. lat. (FAY et al. 2000) and it was even suggested to include this genus (together with *Aphyllanthes*) within *Convallariaceae* (YAMASHITA & TAMURA, 2000). However, our data show that inclusion of *Eriospermum* would result in the loss of bootstrap support for a family *Ruscaceae* s. lat. *Aphyllanthes* was not included in this analysis but *atpB* and *trnL-F* data do not suggest a close relationship to *Ruscaceae* s. lat. (data not shown). Cytological data on the other hand placed *Convallariaceae* s. lat. in vicinity to *Asparagus* but also to *Chlorophytum* (TAMURA 1995).

The recognition of numerous smaller and more homogenous families has been the trend in monocots in general. In the system of DAHLGREN et al. (1985), the concepts of families are very narrow. The single family *Liliaceae* of CRONQUIST's (1981) system is treated in as many as 27 different independent families classified into four orders. However, the results of this and other molecular studies strongly suggest, that the familial status of *Convallariaceae*, *Dracaenaceae*, *Nolinaceae* and *Ruscaceae* s. str. should no longer be recognized. Instead, a larger, highly supported family *Ruscaceae* s. lat. should be recognized which accommodates all members of the four old families. This classification is not only supported by molecular data but also on the basis of a combined molecular-morphological analysis (FAY et al. 2000, RUDALL et al. 2000). The family *Ruscaceae* was erected by SPRENGEL (1826) several years before the family *Convallariaceae* was established by HORANINOW (1834). Because of nomenclatural rules the use of the name *Ruscaceae* s. lat. instead of *Convallariaceae* s. lat. has priority and has to be applied to this group.

One of the major problems in monocot taxonomy has been the proper classification and phylogeny of genera in *Ruscaceae* s. lat. Under the system of DAHLGREN et al. (1985) the genera *Dasyllirion*, *Nolina*, *Beaucarnea* and *Calibanus* were split off as the independent family *Nolinaceae* and were positioned next to *Dracaenaceae*. These two families were placed near the berry-fruited family *Convallariaceae*, with genera

such as *Convallaria*, *Maianthemum*, *Aspidistra*, *Liriope* and near *Asparagaceae* (BOGLER & SIMPSON, 1995). *Nolinaceae* appeared to be much more closely related to genera within *Convallariaceae*, such as *Liriope* and *Maianthemum* than previously suspected, indicating that fruit type, seed characters and chromosome number are good indicators of relationship among these taxa (BOGLER & SIMPSON, 1995). Sequencing data indicated that *Ruscaceae*, *Dracaenaceae* and *Nolinaceae* are related to each other (CHASE et al. 1993; DUVAL et al. 1993), and further support for this relationship came also from cytological data (TAMURA 1995). These three families possess small or medium-sized chromosomes and a basic number of $x = 20$. The basic chromosome number of $x = 19$ in other members of *Ruscaceae* s. lat. might be considered as autapomorphic. Moreover, *Ruscaceae*, *Dracaenaceae*, *Nolinaceae* and *Asparagaceae* may share a common ancestor with $x = 10$ (*Asparagaceae*), from which the basic number of $x = 20$ (*Ruscaceae* and *Dracaenaceae*) was derived through polyploidy, which in turn gave rise to $x = 19$ in *Nolinaceae*. Based on a *rbcL* study, CHASE et al (1995) found the families *Convallariaceae*, *Ruscaceae* s. str., *Dracaenaceae* and *Nolinaceae* to be paraphyletic to each other. In another study RUDALL & CUTLER (1995) also found the *Convallariaceae* being paraphyletic to *Dracaenaceae*, *Eriospermaceae*, *Nolinaceae* and *Ruscaceae*. They even suggested, that *Comospermum* (*Anthericaceae*) fits better into *Convallariaceae* with its berried fruits and flattened rachis. BOGLER & SIMPSON (1995) supposed also *Convallariaceae* s. lat. as a monophyletic group with *Liriope* and *Dracaenaceae* as sister to a clade containing other *Convallariaceae* and *Nolinaceae*. RUDALL (1997) however, regard this grouping as being polyphyletic based on an investigation of microsporogenesis. YAMASHITA & TAMURA (2000) concluded that *Convallariaceae* s. str. are not a monophyletic group and they probably show relationships to allied families. Members of *Convallariaceae* s. str. are rhizomatous perennial herbs and occur in temperate woodland habitats whereas *Ruscaceae* s. lat. extend also into tropical regions of the Northern hemisphere (RUDALL et al. 2000).

Although the tribe Ophiopogoneae traditionally consists of the three genera *Ophiopogon*, *Liriope* and *Peliosanthes* (DAHLGREN et al. 1985, CONRAN 1989), genus *Peliosanthes* did not fall into one clade with *Ophiopogon* and *Liriope* in the *rbcL* and combined analysis. In the *trnL-F* analysis the three genera formed one clade with low bootstrap support. In other studies, the genus *Peliosanthes* was treated as a separate taxon apart from *Ophiopogon* and *Liriope* (NAKAI, 1936; RUDALL et al. 2000). There are several aspects, in which *Peliosanthes* differs from *Ophiopogon* and *Liriope* such as karyotype, leaf anatomy, pollen morphology, and the presence of septal nectaries in *Peliosanthes* (RUDALL et al. 2000). In spite of such differences to other members of *Convallariaceae* sensu DAHLGREN et al. (1985), a closer relationship was supposed by other morphological characters. In *Peliosanthes*, like in other *Convallariaceae*, the filaments fuse into a column surrounding the gynoecium, the corona and stigma are thick and fleshy, more or less filling the narrow floral opening, and the karyotype shows similarity to that of *Aspidistra*. The tribe Ophiopogoneae sensu Dahlgren et al. is supported by karyological characters, with the karyotypes of *Ophiopogon* and *Liriope* being almost identical and being also similar to that of *Peliosanthes* (TAMURA, 1995). Taken together, the phylogenetic position of *Peliosanthes* within *Ruscaceae* s. lat. is still unclear. Characters of *Peliosanthes* also show similarities with *Ruscaceae* like the filaments fusing into a conspicuous column surrounding the ovary (RUDALL & CAMPBELL, 1999).

The phylogenies established here demonstrated the usefulness of *trnL-F* of chloroplast DNA to resolve phylogenetic relationships at lower taxonomic levels. We also used *rbcL* sequence data for comparison at higher taxonomic levels. There was a better resolution in the combined analysis than in those of *rbcL* and/or *trnL-F* alone especially at higher taxonomic levels like Asparagales as a monophyletic group and *Ruscaceae* s. lat. as a group. If monophyly is kept as a criterion for taxonomic groups, then *Ruscaceae* s. str., *Nolinaceae*, *Convallariaceae* and *Dracaenaceae* should be lumped into a larger family *Ruscaceae* s. lat.

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Appendix 1: List of taxa, voucher information, geographic origin, and EMBL database accession numbers for plant materials used in this study. Vouchers are deposited at LI unless otherwise indicated.

Taxon	Family	Origin	Voucher	Literature citation (<i>rbcl</i>)	Literature citation (<i>trnL</i> intron/ <i>trnL-F</i> IGS)
<i>Acorus calamus</i> L.	<i>Acoraceae</i>	cult. B. G. Vienna	PFOSSER M053	DUVALL et al. 1993; M91625	this paper; AJ441159
<i>Asparagus acutifolius</i> L.	<i>Asparagaceae</i>	Greece: Lesbos	SPETA M015	-	this paper; AJ441168
<i>Asparagus falcatus</i> L.	<i>Asparagaceae</i>	cult. B. G. Vienna	JANG L072	-	this paper; AJ441166
<i>Asparagus officinalis</i> L.	<i>Asparagaceae</i>	cult. B. G. Vienna	JANG L043	DUVALL et al. 1993; L05028	this paper; AJ441164
<i>Asparagus tenuifolius</i> L.	<i>Asparagaceae</i>	Slovenia: Lipizza, 390m	SPETA M013	-	this paper; AJ441165
<i>Asparagus</i> sp.	<i>Asparagaceae</i>	Madagascar: Itrcmo Plateau	PFOSSER H630	-	this paper; AJ441167
<i>Asphodelus aestivus</i> REICHB.	<i>Asphodelaceae</i>	-	CHASE 482 (K)	CHASE et al. 2000; Z73682	CHASE et al. 2000; AJ290257/AJ290291
<i>Streptopus amplexicaulis</i> POIR.	<i>Calochortaceae</i>	cult. B. G. Vienna	JANG L065	-	this paper; AJ441161
<i>Streptopus lanceolatus</i> (AIT.) J.L. REVEAL	<i>Calochortaceae</i>	-	KAWANO et al. 03A	SHINWARI et al. 1994; D17381	-
<i>Colchicum byzantinum</i> KER-GAWL.	<i>Colchicaceae</i>	cult. B. G. Vienna	JANG L009	-	this paper; AJ441162
<i>Colchicum speciosum</i> STEVEN	<i>Colchicaceae</i>	cult. B. G. Vienna	JANG L010	CHASE et al. 1993; L12676	this paper; AJ441163
<i>Aspidistra elatior</i> BLUME	<i>Convallariaceae</i>	cult. origin unknown	CHASE 833 (K)	CHASE et al. 1995; Z77269	MEEROW et al. 1999; AF117016/AF117044
<i>Convallaria majalis</i> L. 1	<i>Convallariaceae</i>	Austria: Steiner Felsen	SPETA M027	SHINWARI et al. 1994; D28334	this paper; AJ441180
<i>Convallaria majalis</i> L. 2	<i>Convallariaceae</i>	Austria: Lichtenberg	SPETA M028	SHINWARI et al. 1994; D28334	this paper; AJ441181
<i>Disporopsis pernyi</i> (HUA) DIELS.	<i>Convallariaceae</i>	China: Jiangsu Province	CHASE 493 (K)	FAY et al. 2000; ?	FAY et al. 2000; ?
<i>Liriope platyphylla</i> F.T. WANG & TANG	<i>Convallariaceae</i>	cult. origin unknown	CHASE 131 (NCU)	RUDALL et al. 1997; Z77271	MEEROW et al. 1999; AF117009/AF117038
<i>Maianthemum bifolium</i> (L.) F.W. SCHMIDT.	<i>Convallariaceae</i>	Austria: Lichtenberg, 750m	SPETA M029	-	this paper; AJ441175
<i>Maianthemum dilatatum</i> A. NELSON & MACBRIDE	<i>Convallariaceae</i>	-	CHASE 497 (K)	CHASE et al. 1995; Z77272	-
<i>Ophipogon japonicus</i> KER.	<i>Convallariaceae</i>	cult. B. G. Vienna	JANG L035	-	this paper; AJ441177
<i>Ophipogon</i> sp.	<i>Convallariaceae</i>	Vietnam: E. Hanoi, Ha long Bay	WARZER H595	-	this paper; AJ441176
<i>Ophipogon japonicus</i> var. <i>japonicus</i>	<i>Convallariaceae</i>	-	Unknown	YAMASHITA and TAMURA 2000; AB029841	-
<i>Peliosanthes</i> sp.	<i>Convallariaceae</i>	Indonesia: Borneo	CHASE 847 (K)	RUDALL et al. 1997; Z77273	MEEROW et al. 1999; AF117006/AF117034
<i>Polygonatum hookeri</i> BAKER	<i>Convallariaceae</i>	Nepal	CHASE 492 (K)	CHASE et al. 1995; Z73695	MEEROW et al. 1999; AF117010/AF117036
<i>Reineckea carnea</i> KUNTH.	<i>Convallariaceae</i>	cult. B. G. Vienna	JANG L036	YAMASHITA and TAMURA 2000; AB029834	this paper; AJ441182
<i>Smilacina hondoensis</i> OHWI	<i>Convallariaceae</i>	-	SHINWARI 73	SHINWARI et al. 1994; D17380	-
<i>Smilacina stellata</i> DESF.	<i>Convallariaceae</i>	cult. B. G. Vienna	JANG L058	-	this paper; AJ441174
<i>Dioscorea balcanica</i> KOSANIN	<i>Dioscoreaceae</i>	cult. B. G. Vienna	JANG L059	-	this paper; AJ441160
<i>Dioscorea bulbifera</i> L.	<i>Dioscoreaceae</i>	-	Unknown	KATO et al. 1995; D28327	-

Appendix 1: continued

Taxon	Family	Origin	Voucher	Literature citation (<i>rbcL</i>)	Literature citation (<i>trnL</i> intron/ <i>trnL-F</i> IGS)
<i>Doryanthes excelsa</i> CORREA	<i>Doryanthaceae</i>	Australia: NSW, Brisbane Water N.P., near Mt. White	SPETA H415	CHASE et al. 1995; Z73697	PFOSSER & SPETA 1999; AJ232440/AJ232563
<i>Sansevieria cylindrica</i> BOJ.	<i>Dracaenaceae</i>	-	T. BRADLEY s.n. (GMUF)	CHASE et al. 1993; M96959	-
<i>Sansevieria gracilis</i> N.E. BROWN	<i>Dracaenaceae</i>	cult. B. G. Vienna	JANG L128	-	this paper; AJ441171
<i>Sansevieria metallica</i> GER ET LABR.	<i>Dracaenaceae</i>	cult. B. G. Vienna	JANG L131	-	this paper; AJ441172
<i>Eriospermum bayeri</i> P. L. PERRY	<i>Eriospermaceae</i>	-	UCI ARB 001932	CHASE et al. 1995; Z77277	-
<i>Eriospermum</i> sp. 1	<i>Eriospermaceae</i>	Namibia	SPETA H947	-	this paper; AJ441169
<i>Eriospermum</i> sp. 2	<i>Eriospermaceae</i>	Zimbabwe	PFOSSER M069	-	this paper; AJ441170
<i>Geitonoplesium cymosum</i> A. CUNN.	<i>Hemerocallidaceae</i>	-	Adelaide B.G. 880709	MEEROW et al. 1999; AF116997	MEEROW et al. 1999; AF117027/AF117055
<i>Hemerocallis fulva</i> LINN.	<i>Hemerocallidaceae</i>	-	DUVALL 19920601 (UCR)	DUVALL et al. 1993; L05036	-
<i>Hemerocallis liliosphodelus</i> L.	<i>Hemerocallidaceae</i>	cult. ex B.G. Bonn (410/97)	PFOSSER H435	-	PFOSSER & SPETA 1999; AJ232442/AJ232565
<i>Hemerocallis minor</i> MILL.	<i>Hemerocallidaceae</i>	cult. ex B.G. Bonn (8411/97)	PFOSSER H434	-	PFOSSER & SPETA 1999; AJ2324343/AJ232566
<i>Barnadia scilloides</i> LINDL.	<i>Hyacinthaceae</i>	Japan: ex B.G. Kanagawa (214/94)	PFOSSER H025	SHINWARI et al. 1994; D28161	PFOSSER & SPETA 1999; AJ2324509/AJ232632
<i>Bowiea volubilis</i> HARVEY ex. J.D. HOOKER	<i>Hyacinthaceae</i>	S. Africa, cult. B.G. Vienna	PFOSSER H222	FAY and CHASE 1996; Z69237	PFOSSER & SPETA 1999; AJ232454/AJ232577
<i>Hyacinthus orientalis</i> L.	<i>Hyacinthaceae</i>	Romania:Grecci cult.	SPETA H067	Meerow et al., 1999; AF116995	PFOSSER & SPETA 1999; AJ232539/AF232662
<i>Oziroë biflora</i> (RUIZ & PAV.) F. SPETA	<i>Hyacinthaceae</i>	Chile	MWC 793 (K)	CHASE et al. 1995; Z69222	PFOSSER & SPETA 1999; AJ232453/AF232576
<i>Arthropodium cirrhatum</i> R. Br.	<i>Lomandraceae</i>	-	CHASE 651 (NCU)	FAY and CHASE 1996; Z69233	-
<i>Arthropodium milleflorum</i> (DC) J.F. MACBR.	<i>Lomandraceae</i>	Australia: NSW Barrington TOPS Top N.P. 1100-1400m	SPETA H418	-	PFOSSER & SPETA 1999; AJ232436/AJ232559
<i>Arthropodium</i> sp.	<i>Lomandraceae</i>	Australia: N.S.W., Chichester State Forest, Jerusalem Creek	SPETA H423	-	PFOSSER & SPETA 1999; AJ232435/AJ232558
<i>Thysanotus spiniger</i> BRITTAN	<i>Lomandraceae</i>	-	RUDALL s.n. (K)	FAY and CHASE 1996; Z69236	-

Appendix 1: continued

Taxon	Family	Origin	Voucher	Literature citation (<i>rbcL</i>)	Literature citation (<i>trnL</i> intron/ <i>trnL-F</i> IGS)
<i>Thysanotus virgatus</i> BRITTAN	Lomandraceae	Australia: NSW, Marley Track, Royal N.P.	SPETA H413	-	PFOSSER & SPETA 1999; AJ232437/AJ232560
<i>Calibanus hookeri</i> (LEM.) TREL.	Nolinaceae	Mexico: San Luis Potosi Mts. near Balneario de Lourdes	SCHATZL AG01	RUDALL et al. 1997; Z77276	PFOSSER & SPETA 1999; AJ232434/AJ232557
<i>Dasyllirion acrotrichum</i> ZUCC.	Nolinaceae	cult. B. G. Vienna	JANG L120	-	this paper; AJ441179
<i>Dasyllirion serratifolium</i> ZUCC.	Nolinaceae	-	Unknown	YAMASHITA and TAMURA 2000; AB029847	-
<i>Nolina microcarpa</i> S. WATS	Nolinaceae	cult. B. G. Vienna	JANG L122	-	this paper; AJ441178
<i>Nolina recurvata</i> HEMSL.	Nolinaceae	-	Unknown	YAMASHITA and TAMURA 2000; AB029846	-
<i>Ruscus aculeatus</i> L.	Ruscaceae	Greece: Samos, above Kastanca	SPETA 14399-14403	RUDALL et al. unpublished; Z77274	this paper; AJ441173
<i>Ruscus hypoglossum</i> L.	Ruscaceae	Austria: Reichraminger Hintergebirge	SPETA M019	-	this paper; AJ441158
<i>Bessera elegans</i> Schult. f.	Themidaceae	Mexico: Sinaloa	CHASE 626 (K)	FAY and CHASE 1996; Z69215	MEEROW et al. 1999; AF117015/AF117040
<i>Brodiaea jolonensis</i> EASTW.	Themidaceae	-	CHASE 1831 (K)	FAY et al. 2000; AF116993	MEEROW et al. 1999; AF117017/AF117046
<i>Dichelostemma multiflorum</i> A.A. HELLER	Themidaceae	-	CHASE 1830 (K)	FAY and CHASE 1996; Z69211	-
<i>Dichelostemma pulchellum</i> A. A. HELLER	Themidaceae	cult. ex U of California, Irvine Arboretum	Unknown	-	PIRES et al. 2001; AJ311079
<i>Milla biflora</i> CAV.	Themidaceae	-	CHASE 1907 (K)	FAY and CHASE 1996; Z69216	-
<i>Milla magnifica</i> E. MOORE	Themidaceae	-	MEEROW 2309 (FLAS)	-	MEEROW et al. 1999; AF117011/AF117041

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